IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appellant:	Randall W. Nelson et al.
Serial No.:	09/808,314
Filing Date:	March 14, 2001
Title:	MASS SPECTROMETRIC IMMUNOASSAY
Examiner:	Gary W. Counts
Art Unit:	1641

TO: Mail Stop APPEAL BRIEF-PATENTS
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

APPELLANT'S BRIEF PURSUANT TO 37 C.F.R. § 41.37

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(Submitted in Triplicate)

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PURSUANT TO 37 C.F.R. § 41.37

Dear Commissioner:

Appellant appeals the decision of the Examiner finally rejecting all of the claims pending in the present application, namely claims 31-33, 35-40, 42, 44-46 and 48.

I. REAL PARTY IN INTEREST

Intrinsic Bioprobes, Inc. is the real party in interest in the subject patent application, by virtue of an Assignment from inventors Randall W. Nelson, Peter Williams, and Jennifer Reeve Krone to Intrinsic Bioprobes, Inc. (recorded on June 26, 2004 at Reel 015501, Frame 0555).

II. RELATED APPEALS AND INTERFERENCES

No other appeals or interferences are currently known that will directly affect, be directly affected by, or have a bearing on the decision to be rendered by the Board of Patent Appeals and Interferences in the present Appeal.

III. STATUS OF CLAIMS

Claims 31-33, 35-40, 42, 44-46, and 48 are pending in the application.

Claims 31-33, 35-40, 42, 44-46, and 48 stand rejected under 35 U.S.C. § 103(a) and are appealed herein.

IV. STATUS OF AMENDMENTS

An amendment was filed after the Examiner's Final Office Action, amending claims 31, 33, 37, and 39 and canceling claims 32, 35-36, 38, 40, 42, 45 and 48, without prejudice or disclaimer. The amendment was not entered by the Examiner because the Examiner deemed that the amendment raised new issues that would require further consideration and/or search.

V. SUMMARY OF CLAIMED SUBJECT MATTER

The pending application relates to a method for quantifying the relative amount of one or more analytes (antigens or antibodies) present in a specimen. Antigens or antibodies contained within a specimen are qualitatively and quantitatively determined by utilizing an antibody or antigen to capture and isolate another antigen or antibody, respectively, from its surroundings and then mass spectrometrically analyzing the isolated antibody or antigen after releasing it from the capturing agent. The specificity of the antibody-antigen reaction coupled with the ability of the mass spectrometer to separate and unequivocally identify the captured and isolated antibody or antigen by its mass-to-charge ratio from other molecules that may accompany it lends to two dimensions of specificity. (See specification, pg. 5, lines 21-31).

The quantitative analysis utilizes standard preparations containing known concentrations of the analyte for calibration. Because it is difficult to control the analytical conditions sufficiently well to ensure constant mass spectrometric response for constant analyte concentration in different samples, at least one internal reference species is introduced to the sample prior to incubation with the affinity reagent. The internal reference species may be added to the analytical system being assayed or it may be intrinsic thereto. The internal reference species is captured, isolated and mass spectrometrically analyzed simultaneously with the analyte thereby serving to calibrate the analytical conditions from one analysis to another because both

the analyte and internal reference species respond identically to changes in these conditions. (See specification, pg. 24, lines 23-32 and pg. 25, lines 1-5).

The affinity reagent must contain an affinant that will specifically capture or bind with the internal reference species. (See specification, pg. 25, lines 6-7). The internal reference species is preferably a modified variant of the analyte. An affinant that can capture the analyte can usually also capture the modified variant because the modification shifts the molecular weight of the antibody or antigen without destroying its affinity. Where the internal reference species is not a modified variant of the analyte, another immuno chemical affinity group must be present in the affinity reagent in order to simultaneously capture and isolate the internal reference species along side the analyte. It may be desirable for a protein that is not an antibody to be used as an internal reference standard in an analysis of an antibody species. In such a situation, the affinity reagent would be prepared with two classes of molecules, an antibody specific for the protein and an antigen for which the analyte is specific. (See specification, pg. 25, lines 15-30).

Applicants' claimed method for quantifying the relative amount of one or more analytes present in a specimen includes combining the specimen with a known amount of internal reference species if the specimen does not already contain one, capturing and isolating at least one of the analytes and the internal reference species where the capturing and isolating step includes a substep of combining the internal reference species containing specimen with an affinity reagent, and quantifying the analyte or analytes in which the quantifying step includes using only single dimension mass spectrometric analysis to resolve distinct signals for the analyte and the internal reference species to determine the amount of the captured analyte relative to the internal reference species. (See claim 31). The step of capturing and isolating more than one analyte in the internal reference species includes immobilizing at least one antibody onto a solid substrate to produce an affinity reagent, combining an effective amount of the affinity reagent with the specimen to produce a post-combination affinity reagent and an unbound remainder of the specimen, separating the post-combination affinity reagent from the unbound remainder of the specimen to form an isolated post-combination affinity reagent, and

adding a laser desorption/ionization agent to the isolated post-combination affinity reagent to form a post-combination affinity reagent mass spectrometric mixture. (See claim 32).

VI. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

Claims 31-33, 35 and 36 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Papac et al. (Direct Analysis of Affinity-Bound Analytes by MALDI/TOF, Anal. Chem. 1994, 66, 2609-2613) in view of Gaskell (Quantification of Steroid Conjugates Using Fast Atom Bombardment Mass Spectrometry, Steroids, 1990, Vol. 55, pp. 458-462).

Claims 37-40 and 42 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Papac et al. in view of Gaskell as applied to claims 31-33, 35 and 36 above, and further in view of Chiabrando et al. (Journal of Chromatography 495 (1989) 1-11).

Claims 44-46 and 48 under 35 U.S.C. § 103(a) as being unpatentable over Papac et al. and Gaskell in view of Chiabrando et al. as applied to claims 31-33, 35-40 and 42 above, and further in view of Merren, U.S. Patent No. 3,770,337.

VII. ARGUMENT

The Examiner rejected all of the pending claims as being unpatentable under 35 U.S.C. § 103(a).

To sustain the rejections set out in Section VI above, the Examiner must establish a *prima facie* case of obviousness. Furthermore, the Examiner must establish a *prima facie* case of obviousness by a preponderance of the evidence. The Examiner has not established a *prima facie* case of obviousness because, *inter alia*, (i) the prior art references, taken together, do not teach or suggest all of the elements of Appellant's claims; (ii) there is no suggestion in the prior art to combine or modify all of the references to meet Appellant's claims; and (iii) a combination of the prior art references does not result in Appellant's claims. Because the Examiner has not established obviousness by a preponderance of the evidence, Claims 31-33, 35-40, 42, 44-46 and 48 are patentable over the above cited references. Appellant thus respectfully requests that the rejection of these claims under 35 U.S.C. § 103(a) be withdrawn.

A. **The Examiner Has Not Established a *Prima Facie* Case of Obviousness.**

The Examiner has the initial burden to establish a *prima facie* case of obviousness. To establish a *prima facie* case of obviousness, the Examiner must establish that: (1) the prior art reference (or the references when combined) teaches or suggests all the elements of Appellant's claims; and (2) there is some suggestion, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to combine or modify the references. In the present case, the Examiner has failed to establish that the Papac and Gaskell references recite all of the claim elements including, *inter alia*, the step of quantifying one or more analytes using only single dimension spectrometric analysis to resolve distinct signals for the analyte and the internal reference species to determine the amount of the captured analytes relative to the internal reference species. Moreover, there is no suggestion in any of the prior art references cited by the Examiner (or elsewhere in the prior art) to combine or modify the references to achieve Appellant's claims. Thus, the Examiner has failed to establish a *prima facie* case of obviousness for Applicants' claims.

To establish a *prima facie* case of obviousness, the Examiner must show either how the prior art references suggest, either expressly or impliedly, the combination that results in Appellant's claims or, alternatively, the Examiner must present a convincing line of reasoning as to why one skilled in the art would have found the claims to have been obvious in light of the teachings of the references. *Ex parte Clapp*, 227 U.S.P.Q. 972, 973 (Bd. of Pat. Appeals and Interferences, 1985). When the motivation to combine the teachings of the prior art references is not immediately apparent, it is the duty of the Examiner to explain why the combination of the teachings is proper. *Ex parte Skinner*, 2 U.S.P.Q.2d 1788 (Bd. of Pat. Appeals and Interferences, 1986). Significantly, the fact that references can be combined or modified does not render the resultant combination obvious unless the prior art suggests the desirability of the combination. *In re Mills*, 916 F.2d 680 (Fed. Cir., 1990). The teaching or suggestion to make a claimed combination must be found in the prior art and must not be based on Appellant's disclosure. *In re Vaeck*, 947 Fed.2d 488 (Fed. Cir., 1991).

The test of obviousness is not whether features of a secondary reference may be bodily incorporated into a primary reference's structure, nor whether a claimed invention is expressly suggested in any one or all of the references. Instead, the test is what the combined teachings of references would have suggested to those of ordinary skill in the art. *In re Keller, Terry, and Davies*, 208 U.S.P.Q. 871, 881 (C.C.P.A., 1981). The mere fact that the prior art may be modified to reflect features of the claimed invention does not make the modification, and hence the claimed invention, obvious unless the desirability of such a modification is suggested by the prior art. The claimed invention cannot be used as an instruction manual or "template" to piece together teachings of the prior art so that the claimed invention is rendered obvious. *In re Fritsch*, 23 U.S.P.Q.2d 1780, 1783-84 (C.A.F.C., 1992).

1. Claims 31-33, 35 and 36.

The Examiner rejected Claims 31-33, 35 and 36 under 35 U.S.C. §103(a) as being unpatentable over Papac et al. (Analytical Chemistry) in view of Gaskell (Quantification of Steroid Conjugates Using Fast Atom Bombardment Mass Spectrometry, Steroids, 1990, Vol. 55, pages 458-462). In particular, the Examiner states that Papac discloses a method for the mass spectral identification and detection of analytes separated by immunoaffinity chromatography, using antibody immobilized agarose beads as affinity columns, combining a specimen with the beads to capture antigen present in the sample (post-combination affinity reagent), washing to remove any unbound antigen, mixing sample with the beads and centrifuging and removing supernatant, adding a matrix containing formic acid to the supernatant and testing by MALDI-TOF mass spectrometry (single dimension mass spectrometric analysis), and determining the analyte by mass-to-charge ratio. Although the Examiner concedes that Papac fails to teach that the specimen is combined with an internal reference species of known concentration prior to capturing and isolating the analyte and IRS, and also failing to teach quantifying the analyte, the Examiner contends that Gaskell discloses quantifying an analyte where a deuterated internal standard is added to the sample which is then mixed with the solid phase incorporating bound antiserum for isolating the analyte and internal standard. The Examiner further contends that Gaskell discloses that for quantification of the analyte, the analyte and the internal standard are compared to a standard curve and that the standard curve was obtained by analysis of standard

mixtures of the analyte and analyte analog. The Examiner also asserts that Gaskell discloses that the addition of an internal standard provides for precise and accurate data and provides for the quantification of an analyte. Accordingly, the Examiner contends that it would have been obvious to one of ordinary skill in the art to incorporate an internal standard and affinity reagent and also to develop a standard curve for quantification analysis into the method of Papac because Gaskell teaches that the addition of an internal standard provides for precise and accurate data and provides for the quantification of an analyte of interest. The Examiner further contends that one of ordinary skill in the art would have had a reasonable expectation of success by incorporating an internal standard and affinity reagent as taught by Gaskell into the method of Papac. Applicants respectfully traverse this rejection.

First, unlike Applicants' claimed invention, the Papac reference analyzes a predetermined analyte and not the identification and amount of analyte present in a biological or physiological specimen. In addition, in Papac, aliquots of beads containing the predetermined analyte are removed from the column for performing MALDI/TOF analysis (see page 2611, column 1, first paragraph). The discussion under mass spectrometry on page 2611, first paragraph of the Papac reference, merely describes how the same aliquots of beads containing the known analytes were prepared for performing mass spectrometry. Further, this is clearly confirmed in the results and discussion section which states: "Purification is necessary before binding the antibody to the affinity support. To accomplish this purification, Cytochrome C was first bound to the affinity support (see experimental section). The crude antibody solution was passed through the column, and a 1 microliter aliquot of the column bed was used to acquire the MALDI/TOF spectrum shown in Figure 1A" (Papac, pg. 2611, bottom of column 1, top of column 2). In the Applicants' claimed invention, a released analyte species is detected using a mass spectrometer to determine whether the analyte species is present in the physiological specimen, determining the identity of the analyte species using molecular weight analysis, and determining the quantity of the analyte species using the internal reference species. The Papac reference fails to disclose any quantification whatsoever of the analyte.

Second, the Gaskell reference cited by the Examiner discloses fast atom bombardment/mass spectrometry or liquid secondary ion mass spectrometry to analyze steroid

conjugates (sulfates, gulcuronides) without prior hydrolysis or derivitization. In particular, the Gaskell reference describes the quantitative determination of dehydroepiandrosterone sulfate in serum by selective isolation of the analyte using immunoadsorption extraction and highly specific detection using tandem mass spectrometry. The quantification method includes 1) stable isotope dilution using an internal standard, 2) isolation of the analyte by immunoadsorption, and 3) detection of both the analyte and internal standard during limited mass range parent ion scanning during tandem mass spectrometry (see page 460, column 1, fourth paragraph of the Gaskell reference). Furthermore, the Gaskell reference specifically states that "the success of the detection procedure was dependent both on the selectivity of tandem MS detection and on the achievement of a sufficiently "clean" biologic extract by immunoadsorption." (See page 461, column 2, second paragraph of Gaskell). Accordingly, the Gaskell reference cited by the Examiner actually teaches away from the instantly claimed invention by using tandem MS for quantification. In other words, different mass spectrometric measurements were taken of similar portions of the same serum extract and compared. In contrast, in Applicants' instantly claimed invention, the analyte and IRS are measured using MS in a single measurement, i.e. single dimension mass spectrometry. Accordingly, it would not have been obvious to one of ordinary skill in the art to incorporate the method disclosed in Gaskell into the method of Papac to arrive at Applicants' claimed invention because Applicants' claimed invention would then require tandem MS. In contrast, Applicants' claimed invention requires single dimension MS.

2. Claims 37-40 and 42.

The Examiner rejected Claims 37-40 and 42 under 35 U.S.C. §103(a) as being unpatentable over Papac et al. in view of Gaskell as applied to claims 31-33, 35 and 36 above, and further in view of Chiabrando et al. (Journal of Chromatography). In particular, although the Examiner concedes that Papac and Gaskell fail to teach combining a plurality of distinctive internal reference species to a sample, the Examiner contends that Chiabrando discloses adding multiple deuterated internal standards to a sample and also using immobilized antibodies to capture and isolate the analytes and internal standards. The Examiner further contends that Chiabrando discloses that this provides for the simultaneous measurement of analytes and their metabolites. Accordingly, the Examiner contends that it would have been obvious to one of

ordinary skill in the art to incorporate multiple internal standards at taught by Chiabrando into the modified method of Papac because Chiabrando discloses that this provides for the simultaneous measurement of analytes and their metabolites and further because it would have been obvious to one of ordinary skill in the art to use different types of standards with the different analytes to be detected. Applicants' respectfully traverse this rejection.

Papac fails to disclose the use of combining an internal reference species with a specimen, capturing and isolating an analyte and the internal reference species contained in the specimen, and quantifying the analyte using single dimension mass spectrometric analysis to resolve signals for the analyte and the internal reference species to determine the amount of the captured analyte. Further, as previously set out above, the Gaskell reference fails to disclose using single dimension mass spectrometry and instead requires using tandem mass spectrometry for detecting an analyte and internal standard and for quantifying the analyte. Therefore, it could not have been obvious to one of ordinary skill in the art to combine Gaskell and Papac to arrive at Applicants' claimed invention which utilizes single dimension mass spectrometry to resolve distinct signals for the analyte and the IRS to determine the amount of captured analyte. Chiabrando discloses a method which utilizes gas chromatography-mass spectrometry. Chiabrando fails to disclose the use of single dimension mass spectrometry to analyze and quantify an analyte. Therefore, it would not have been obvious to one of ordinary skill in the art to combine Chiabrando with Papac and Gaskell to arrive at Applicants' claimed invention.

3. **Claims 44-46 and 48.**

The Examiner rejected Claims 44-46 and 48 under 35 U.S.C. §103(a) as being unpatentable over Papac et al. and Gaskell in view of Chiabrando as applied to claims 31-33, 35-40 and 42 above, and further in view of Merren. Although the Examiner concedes that Gaskell and Chiabrando fail to specifically teach interpolating the analyte species mass spectrometric response to the IRS's mass spectrometric response, the Examiner contends that Merren teaches the addition of a reference substance which provides a spectrum containing peaks at several known mass-to-charge ratios. The Examiner further contends that Merren teaches that the reference spectrum is accurately correlated with the spectrum of the unknown substance and that the reference peaks therefore act as accurate markers forming a calibrated scale from which the

mass-to-charge ratios of peaks of the unknown substance are interpolated. The Examiner further states that Merren teaches that this provides a method for combining signals representative of the simultaneous spectral analysis of two substances, thereby permitting single channel processing of the combined signal. Accordingly, the Examiner contends that it would have been obvious to one of ordinary skill in the art to interpolate the analyte species in the reference species as taught by Merren into the modified method of Papac because Merren shows that this provides a method for combining signals representative of the simultaneous spectral analysis of two substances thereby permitting signal channel processing of the combined signal. Applicants respectfully traverse this rejection.

As previously stated above, it would not have been obvious to one of ordinary skill in the art to combine Papac, Gaskell, and Chiabrando to arrive at claims 31-33, 35-40 and 42 and those arguments are herein incorporated by reference in their entirety.

Furthermore, Merren discloses a double beam mass spectrometer for simultaneously enabling mass spectral analysis for two substances such as an unknown and a reference substance. Merren fails to disclose single dimension mass spectrometric analysis of an analyte and an internal reference species using a standard single beam mass spectrometer. Therefore, it would still not have been obvious to one of ordinary skill in the art to combine Merren with Papac and Gaskell to arrive at Applicants' claimed invention. Accordingly, it would not have been obvious to one of ordinary skill in the art to combine Merren with Papac, Gaskell, and Chiabrando to arrive at Applicants' claims 44-46 and 48.

With respect to the Examiner's response to Applicants' arguments and the Examiner's statement that the Examiner has not relied upon Gaskell for teaching tandem MS but has instead relied upon Gaskell for teaching that it is known in the art to incorporate internal references into a sample for the quantification of an analyte, Applicants reassert their argument that it would not have been obvious to one of ordinary skill in the art to combine Gaskell with Papac because Gaskell is directed to using an internal reference standard with tandem MS. Accordingly, the combination of Papac and Gaskell cannot teach a single dimension mass spectrometric process for quantifying an analyte using internal reference species.

B. The Examiner Has Not Established a *Prima Facie* Case of Obviousness By a Preponderance of the Evidence.

As mentioned above, the Examiner has the initial burden of factually supporting a *prima facie* case of obviousness. This has not been done. Additionally, the Examiner must prove her case by a preponderance of the evidence, with due consideration to the persuasiveness of any arguments in rebuttal. In re Attacher, 977 F.2d 1443 (Fed. Cir., 1992). When the motivation to combine the teachings of the prior art references is not immediately apparent, it is the duty of the Examiner to explain why the combination of the teachings is proper. Ex parte Skinner, 2 U.S.P.Q.2d 1788 (Bd. of Pat. Appeals and Interferences, 1986). Moreover, the fact that references can be combined or modified does not render the resultant combination obvious unless the prior art suggests the desirability of the combination and/or modification. In re Novo, 916 F.2d 680 (Fed. Cir., 1990). Furthermore, the Examiner cannot suggest the combination or modification based on hindsight reconstruction.

As shown above, the Examiner has failed to meet his burden of persuasion. The Examiner did not identify any express or implied suggestion in either the Papac, Gaskell, Chiabrando, or Merren references (or anywhere else) to combine or modify the references. Furthermore, although the Examiner stated why he believed it would be obvious to one skilled in the art to modify combine the Papac and Gaskell references, it was shown that such a combination would not arrive at Appellant's method because neither reference discloses quantification of an analyte using single dimension mass spectrometry. Therefore, the Examiner failed to provide a "convincing line of reasoning". Instead, the Examiner's suggestion and modification of the combined references resulted in quintessential hindsight reconstruction. Therefore, because the Examiner did not establish by a preponderance of the evidence a *prima facie* case of obviousness, Appellant respectfully submits that the associated rejections of Claims 31-33, 35-40, 42, 44-46 and 48 under 35 U.S.C. § 103(a) should be withdrawn.

VIII. CONCLUSION

For the above reasons, Claims 31-33, 35-40, 42, 44-46 and 48 fully comply with 35 U.S.C. § 112 and are not obvious to one skilled in the art having knowledge of the Papac,

Gaskell, Chiabrando and Merren references. Accordingly, Appellant respectfully submits that Claims 31-33, 35-40, 42, 44-46 and 48 are patentable over the prior art and respectfully requests this Board to so indicate.

Applicants authorize and respectfully request that the $250.00 filing fee for the filing of this Appeal Brief be charged to Snell & Wilmer's Deposit Account No. 19-2814. **A duplicate copy of this document is attached.**

Dated: July 7, 2006

Respectfully submitted,

By _____
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IX. CLAIMS APPENDIX

31. A method for quantifying the relative amount of one or more analytes present in a specimen, comprising the steps of:

a. combining said specimen with a known amount of internal reference species (IRS) if the specimen does not already contain one;

b. capturing and isolating at least one of the one or more analytes and said IRS, wherein said capturing and isolating step comprises a substep of combining said IRS containing specimen with an affinity reagent;

c. quantifying the at least one of the one or more analytes in which said quantifying step comprises using only single dimension mass spectrometric analysis to resolve distinct signals for the analyte and said IRS to determine the amount of the captured analytes relative to the IRS.

32. The method according to claim 31 in which said capturing and isolating step further comprises the steps of:

a. immobilizing at least one antibody onto a solid substrate to produce an affinity reagent;

b. combining an effective amount of the affinity reagent with the specimen to produce a post-combination affinity reagent and an unbound remainder of the specimen;

c. separating the post-combination affinity reagent from the unbound remainder of the specimen to form an isolated post-combination affinity reagent;

d. adding a laser desorption/ionization agent to the isolated post-combination affinity reagent to form a post-combination affinity reagent mass spectrometric mixture.

33. The method according to claim 32 in which said quantifying step further comprises the steps of:

a. mass spectrometrically analyzing the post combination affinity reagent mass spectrometric mixture to produce a post combination affinity reagent mass spectrum

having a mass spectrometric response for the internal reference species located at a unique mass-to-charge ratio of the IRS, and an analyte mass spectrometric response as a unique mass-to-charge ratio of each analyte species thereby detecting the analyte species and no mass spectrometric response corresponding to the mass-to-charge ratio of the analyte species when the specimen contains no detectable amount of the analyte species; and

b. determining whether the amount of the analyte species present in the sample is greater or less than the known amount of the IRS by comparing the mass spectrometric response for detected analyte species relative to the mass spectrometric response for the IRS.

35. The method of claim 32 further including the step of adding a disassociation agent to the isolated post-combination affinity reagent prior to the adding laser desorption/ionization agent step.

36. The method of claim 33 further including the step of adding a disassociation agent to the isolated post-combination affinity reagent prior to the adding laser desorption/ionization agent step.

37. A method for quantifying the relative amount of one or more analytes present in a specimen, comprising the steps of:

a. combining said specimen with a plurality of distinctive internal reference species (IRS's) which correspond to the one or more analytes in the specimen in varied and known concentrations, each of the concentrations being chosen to produce a different mass spectrometric response after mass spectrometric immunoassay;

b. capturing and isolating at least one of the one or more analytes and said plurality of IRS's, wherein said capturing and isolating step comprises a substep of combining said plurality of IRS's containing specimen with an affinity reagent;

c. quantifying the at least one of the one or more analytes in which said quantifying step comprises using only single dimension mass spectrometric analysis to resolve

distinct signals for the analyte and said IRS's to determine the amount of the captured analytes relative to the IRS's.

38. The method according to claim 37 in which said capturing and isolating step further comprises the steps of:

a. immobilizing at least one antibody onto a solid substrate to produce an affinity reagent;

b. combining an effective amount of the affinity reagent with the specimen to produce a post-combination affinity reagent and an unbound remainder of the specimen;

c. separating the post-combination affinity reagent from the unbound remainder of the specimen to form an isolated post-combination affinity reagent;

d. adding a laser desorption/ionization agent to the isolated post-combination affinity reagent to form a post-combination affinity reagent mass spectrometric mixture.

39. The method according to claim 38 in which said quantifying step further comprises the steps of:

a. mass spectrometrically analyzing the post combination affinity reagent mass spectrometric mixture to produce a post combination affinity reagent mass spectrum having a mass spectrometric response for the plurality of IRS's located at a unique mass-to-charge ratio of the IRS's, and an analyte mass spectrometric response at a unique mass-to-charge ratio of each analyte species thereby detecting the analyte species and no mass spectrometric response corresponding to the mass-to-charge ratio of the analyte species when the specimen contains no detectable amount of the analyte species; and

b. determining whether the amount of the analyte species present in the sample is greater or less than each of the known amounts of the plurality of IRS's by comparing the mass spectrometric response for detected analyte species relative to the mass spectrometric response for the plurality of IRS's.

40. The method of claim 38 further including the step of adding a disassociation agent to the isolated post-combination affinity reagent prior to the adding laser desorption/ionization agent step.

42. The method of claim 39 further including the step of adding a disassociation agent to the isolated post-combination affinity reagent prior to the adding laser desorption/ionization agent step.

44. The method according to claim 37 in which said quantifying step further comprises interpolating each of the analyte species mass spectrometric response to the plurality of IRS's mass spectrometric response immediately above and below in magnitude of each of the IRS's mass spectrometric response to quantify each the analyte species in the specimen.

45. The method according to claim 38 in which said quantifying step further comprises interpolating each of the analyte species mass spectrometric response to the plurality of IRS's mass spectrometric response immediately above and below in magnitude of each of the IRS's mass spectrometric response to quantify each the analyte species in the specimen.

46. The method according to claim 39 in which said quantifying step further comprises interpolating each of the analyte species mass spectrometric response to the plurality of IRS's mass spectrometric response immediately above and below in magnitude of each of the IRS's mass spectrometric response to quantify each the analyte species in the specimen.

48. The method according to claim 40 in which said quantifying step further comprises interpolating each of the analyte species mass spectrometric response to the plurality of IRS's mass spectrometric response immediately above and below in magnitude of each of the IRS's mass spectrometric response to quantify each the analyte species in the specimen.

X. EVIDENCE APPENDIX

None

XI. RELATED PROCEEDINGS APPENDIX

None